2. *Synthesis and Characterization of Novel Stationary Phases for Liquid Chromatography*

***L.C. Sander**, M. Pursch, and J. Wegman (Univ. of Tuebingen, Tuebingen, Germany)*

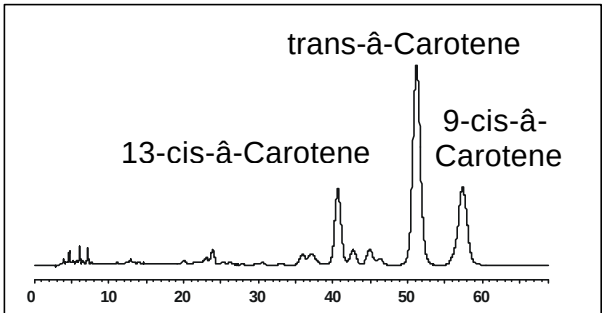
Objective: To enhance NIST capabilities for determination of isomeric species in natural-matrix samples through development of novel chromatographic stationary phases.

Problem: The liquid chromatographic (LC) separation of certain classes of isomers can present a significant challenge to the analyst. In addition to polycyclic aromatic hydrocarbons (PAHs), carotenoids and certain other nutrients are examples of compounds that occur in highly isomeric mixtures. The complexity of these mixtures is increased by other sample matrix elements, adding to the difficulty of analyses. Because the biological activity of contaminants and nutrients differ with molecular structure, measurement of individual isomers is required for assessment of potential adverse or beneficial health effects. Conventional commercial C_{18} columns do not exhibit sufficient inherent selectivity toward shape-constrained isomers to permit determination of many such species.

Approach: In recent years, research efforts within the Organic Analytical Methods Group have been directed toward improving analytical measurement capability for carotenoids, retinoids, tocopherols, and related nutrients in foods and blood serum. Part of this effort has involved the synthesis and characterization of novel LC stationary phases with enhanced selectivity toward carotenoid isomers. A highly successful result of this research has been the development of a column based on a polymeric C_{30} surface modification scheme. Further improvements in column performance are anticipated by the development of stationary phases based on longer alkyl chain lengths. Unfortunately, suitable reagents to prepare such long chain length stationary phases are not commercially available and cannot be synthesized in practical quantities or sufficient purity. To overcome these difficulties we have utilized a novel approach to surface modification involving immobilization of a poly(ethylene-co-acrylic acid) polymer by chemical and physical means.

Results and Future Plans: A series of stationary phases were prepared with poly(ethylene-co-acrylic acid) polymers with different composition. Three approaches to immobilization were studied: aminopropyl linkages, glycidoxypropyl linkages, and physical immobilization (i.e., no chemical bond). Columns were prepared and characterized by use of SRM 869 "Column Selectivity Test Mixture," candidate SRM 870 "Column Performance Test Mixture," and various carotenoid isomer mixtures. Initial results are extremely encouraging. Columns exhibited high chromatographic efficiency with symmetric peak shape, and retention behavior was highly shape-selective. Most importantly, separation of several important carotenoid isomers was achieved with the new polyethylene columns (Cf. figure).

The results shown represent our first efforts using this new synthetic approach. Optimization of column performance will require further study of the reaction parameters, including polyethylene copolymer composition, reagent concentration, type of covalent linkage, choice of silica, and reaction time. It is anticipated that the improved selectivity exhibited by polyethylene stationary phases will help to eliminate measurement biases for carotenoids that result from component coelution.



Separation of carotenoid isomers with a polyethylene stationary phase.